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Many organic pollutants potentially are available for uptake by plants and thus bioaccumulation and food contamination. One method of studying uptake is with excised roots, a technique extensively used with plant nutrients. A similar method was developed and used to evaluate uptake patterns of several ^{14}C-labeled organic chemicals. Uptake rate constants for the chemicals tested occurred in the following order: captan $\simeq$ phenol $>$ aniline $>$ ethanol $\simeq$ indole $\simeq$ trifluralin $\simeq$ propanil $>$ 1,2,4-trichlorobenzene(TCB) $>$ nitrobenzene $\simeq$ atrazine $>$ bromacil $>$ simazine $>$ monuron.

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EXCISED BARLEY ROOT UPTAKE OF SEVERAL ^{14}C LABELED ORGANIC COMPOUNDS

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Abstract. Many organic pollutants potentially are available for uptake by plants and thus bioaccumulation and food contamination. One method of studying uptake is with excised roots, a technique extensively used with plant nutrients. A similar method was developed and used to evaluate uptake patterns of several ^{14}C-labeled organic chemicals. Uptake rate constants for the chemicals tested occurred in the following order: captan ≃ phenol > aniline > ethanol ≃ indole ≃ trifluralin ≃ propanil > 1,2,4-trichlorobenzene (TCB) > nitrobenzene ≃ atrazine > bromacil > simazine > monuron.

1. Introduction

Many organic pollutants are released into terrestrial environments and potentially are available for plant uptake and thus bioaccumulation and food contamination. Therefore it is important to understand the biological, physical and chemical properties which determine organic chemical uptake in plants.

Most previous work on chemical uptake kinetics in plants has emphasized electrolytes associated with plant nutrition, relatively long-lived radionuclides, and heavy metals of ecological importance. As a result there is an extensive body of information on many aspects of uptake and transport of minerals in plants (i.e. Pitman, 1976, 1977; Anderson, 1976; Epstein, 1972; Laties, 1969; Clarkson, 1974; Wardlaw and Passioura, 1976). There also is considerable information on the symplastic movement of plant sugars, organic acids and hormones. However, there is scant information on uptake and/or movement of exogenous organic chemicals in plants.

The objective of these experiments was to develop a procedure for studying toxic organic chemical uptake and to collect information on uptake rates of various chemicals by plant roots.

2. Methods

Barley (*Hordeum vulgare*, cv. Klages) roots were obtained by growing seeds on a double layer of cheesecloth supported by a stainless steel screen (8-mesh, 20 gauge) over a container of half-strength modified Hoagland nutrient solution (Berry, 1978). The cheesecloth was draped over the ends of the screen to provide water and nutrients by capillarity. The solution was replaced on the third day. Since the roots were then long enough to be immersed, the trailing edges of cheesecloth were cut off. On the seventh day, the roots were harvested by cutting between the screen and the cheesecloth with

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an electric knife. Approximately 0.5 g portions of roots were enclosed in baskets made of stainless steel screen (6.3 × 3.5 × 2.5 cm, 8-mesh, 20 gauge). Nine treatment baskets were arranged on a platform made of stainless steel wire mesh. The baskets, containing roots, were submerged in nutrient solution until treatment started (generally within 2 to 10 min).

Treatment was started by transferring the platform and its contents into the treatment tank which contained 2.0 l of nutrient solution and the ^{14}C-labeled test chemical. Baskets containing roots were sequentially removed from the treatment solution at 3 min intervals and submerged in nutrient solution without the chemical for 30 sec to rinse the test chemical from the exterior surfaces. Solutions in the treatment and rinse tanks were stirred by two magnetic bars below the mesh platform. The root sample was removed from the basket, blotted between two sponges and put into a paper thimble (a container used for sample oxidation). Most test chemicals had low vapor pressures and were freeze-dried and later analyzed for ^{14}C content. When the test chemical had a relatively high vapor pressure, the thimbles were put into small closed vials to prevent evaporation and were oxidized fresh as soon as possible (always within 15 min). In an earlier experiment, the nitrobenzene concentration in barley roots exposed to the ^{14}C-labeled compound was found to be the same in roots oxidized after freeze-drying as in those oxidized fresh. Root samples exposed to chemicals with vapor pressures lower than that of nitrobenzene were freezed-dried while the others were oxidized fresh. The CO_2 generated by sample oxidation was collected in a liquid scintillation cocktail and the ^{14}C activity was determined in a liquid scintillation counter (Wickliff *et al.*, 1984).

These tests were done using chemical concentrations within an expected environmental range (generally 10 mg l^{-1} or less). In most cases, the radio-labeled test chemical was diluted with unlabeled test chemical and dissolved in ethanol before adding to the nutrient solution. The nominal solution concentrations of 1,2,4-TCB, indole, ethanol, aniline, and phenol were calculated from the chemical amounts supplied by each chemical source and the amount of unlabeled chemical used in each test. All other test chemicals were analyzed in the treatment solution by high performance liquid chromatography (HPLC) or gas-liquid chromatography (GLC).

At times, it was difficult to start the tests at exactly the same time, relative to cutting the roots. The start times varied from two to about ten minutes after cutting. This caused the researchers to question the possibility of changes in root viability and the effect of the time between cutting the roots and testing uptake on the uptake rate. This was investigated as a quality assurance measure using bromacil as the representative chemical. The results (Figure 1) show that uptake for a 3-min period was not affected by the time since cutting the root tissue (within the limits of this test).

3. Results and Discussion

Rate constants were determined by dividing the measured ^{14}C activity in roots (expressed as DPM/g dry weight) by the solution activity (DPM/ml) and then differ-

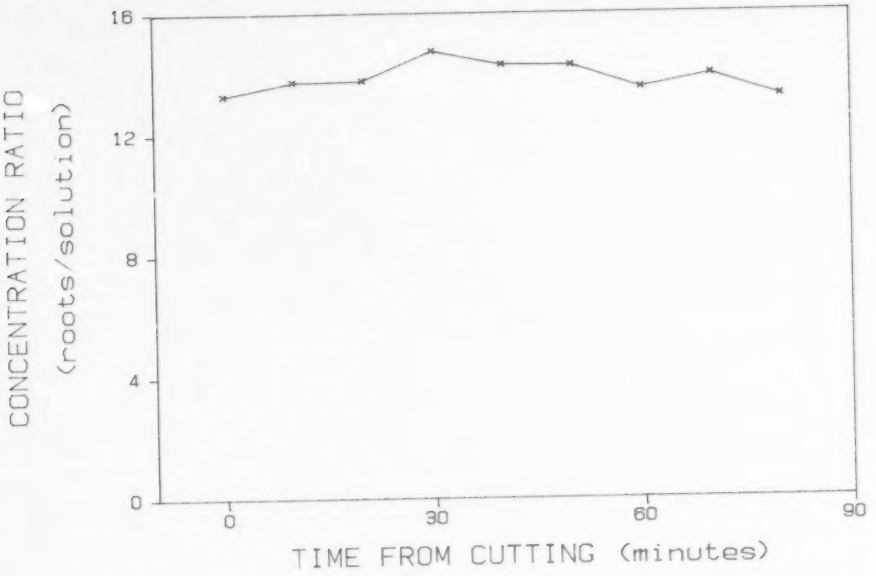

Fig. 1. Bromacil concentration in barley roots after 3 min exposures. Root treatments started at 10 min intervals to 80 min.

entiating with respect to time. The slope (rate constant) represents the fractional chemical uptake from solution to roots per minute. A typical data set is shown for indole in Figure 2.

The validity of a rate constant assumes that chemicals act independently. This is true in dilute solutions, however at high concentrations chemicals interact competitively, generally decreasing chemical activity and thus decreasing diffusion rates and other similar functions. A series of tests were done to verify that the solution concentrations used were dilute enough to be acceptable and that identifying a rate of initial uptake as a constant was justified.

Atrazine, bromacil, and nitrobenzene were each tested at several different concentrations. Atrazine was tested 6 times with the lowest solution concentration being 0.004 and the highest 0.97 mg l^{-1} (Table I). There were no significant differences between rate constants at different chemical concentrations. Furthermore no correlation was observed between rate constant and treatment concentration. Thus, the use of the term constant was justified.

Chemical uptake by excised root tissue, whether passive or active, is a dynamic process which generally attains equilibrium. At equilibrium, the rate of influx equals the rate of efflux and no net transfer of chemical occurs. This can be observed if the treatment period is prolonged (i.e. Figure 3). The kinetics of this system are properly described by an exponential growth function:

$$y = a(1 - e^{-bt})$$

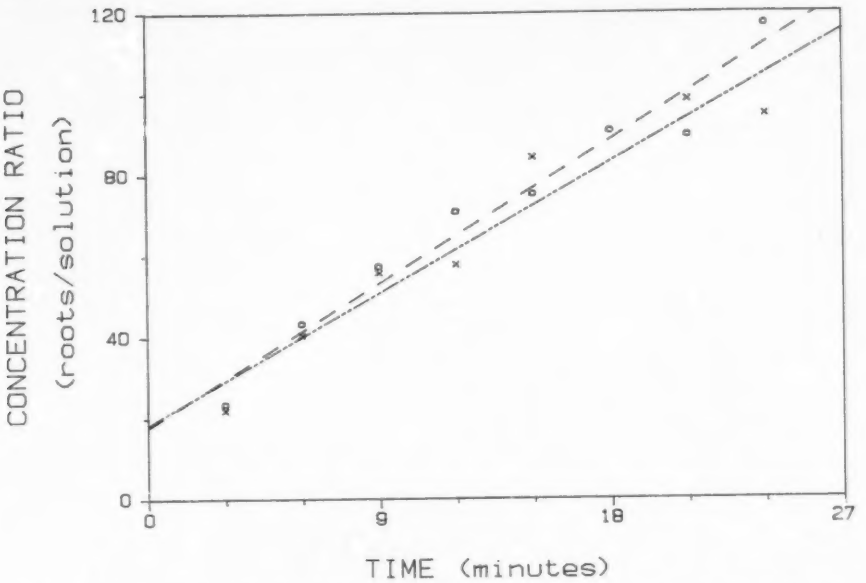

Fig. 2. Indole uptake by excised barley roots. Labeled and unlabeled indole were mixed to produce a treatment solution which was 1.0 mg l^{-1} indole and 1000 DPM ml^{-1} C-14 activity. Two lines (o - - - - - o, and x ——— x) represent separate replications. The slope represents the chemical uptake rate constant.

TABLE I

Chemical uptake rate constants using several different solution concentrations

Chemical	Rate constant (min^{-1})	Std. error of slope (min^{-1})	Solution conc.[a] ($mg\ l^{-1}$)
Atrazine	0.8	0.12	0.12
	0.9	0.05	0.97
	1.1	0.10	0.004
	1.1	0.08	0.11
	1.2	0.09	0.12
	1.3	0.10	0.004
Bromacil	0.6	0.07	5.9
	0.7	0.08	10.0
	0.8	0.07	0.77
Nitrobenzene	1.0	0.09	11.0
	1.2	0.11	9.9
	1.3	0.10	11.0
	1.5	0.13	0.19

[a] Analysis done with HPLC and GLC.

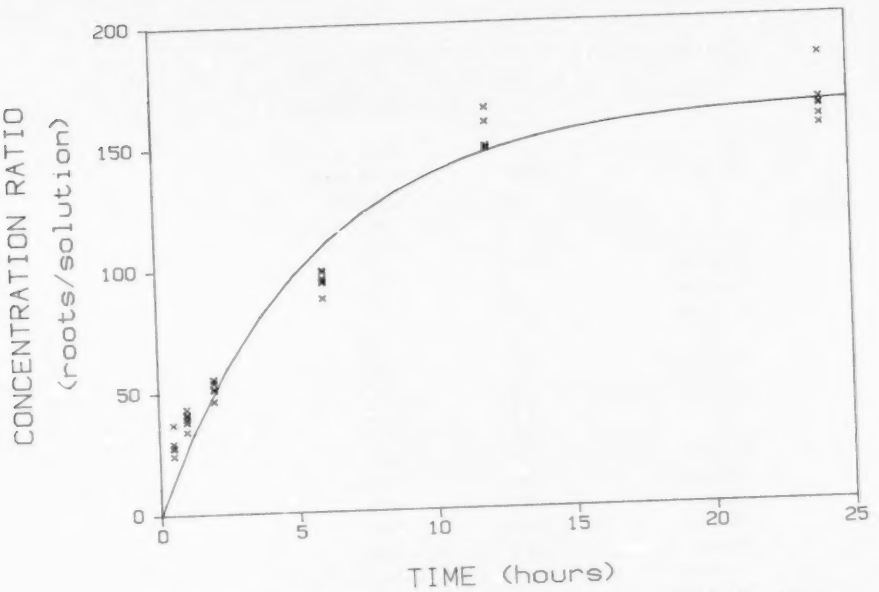

Fig. 3. Nitrobenzene uptake over a 24-hr period. Equilibrium is represented by the plateau.

where a is the maximum value of y (concentration ratio of chemical in the roots divided by chemical concentration in the treatment solution); b is the uptake rate parameter (time^{-1}); and t is time in appropriate units (minutes or hours). With many organic chemicals, equilibrium was reached after about 8 to 12 hr (Wickliff *et al.*, 1984, and unpublished data). Experiments of this duration are informative in some respects, but the prime concern is the maximum uptake rate which can only be evaluated at the limit ($t = 0$), i.e. when there is no efflux of chemical from the roots. This is observed within the first few minutes of the uptake period. Although not truly linear, as explained above, the initial uptake of organic chemicals can be evaluated best and most easily by determining the slope over the first 30 min of uptake using a linear regression analysis (Figure 2). Frequently the chemical concentration in the first sample (3-min sample) was higher than could be explained by the subsequent uptake curve (curve did not go through the origin). This is thought to occur because of surface adsorption, a process which is completed after the first few minutes.

The tested chemicals listed in Table II are arranged in order of increasing rate constants. These data represent a relative ranking for uptake potential by excised plant roots of chemicals dissolved in an aqueous solution.

The data in the tables do not represent the uptake of these chemicals from soil into whole, intact plants. That interaction is certainly more complex and depends on phenomena such as soil binding, plant transpiration, root respiration, and probably photosynthesis and the activity of various enzyme systems. Nevertheless, these data

TABLE II

Chemical uptake rate constants in excised barlev roots. Rate constants are the means of two replicate tests (except those for monuron and simazine, which are based on one test)

Chemical	Rate constant (min^{-1})	Std. error of slope (min^{-1})
Monuron	−0.24[a]	0.08[a]
Simazine	0.25[a]	0.04[a]
Bromacil	0.7	0.04
Atrazine	1.1	0.04
Nitrobenzene	1.2	0.05
1,2,4-TCB	2.2	0.10
Propanil	3.0	0.10
Indole	3.8	0.10
Ethanol	3.9	0.13
Trifluralin	5.0	0.20
Aniline	12.5	0.24
Phenol	33.8	0.32
Captan	34.5	0.28

[a] One test.

represent a valid plant/chemical interaction which forms part of the complete story of chemical-plant kinetics. This relative ranking (when expanded) may yield a corollary to chemical structure which will lead to understanding the controlling mechanisms.

This procedure is a quick, inexpensive, yet reliable test method useful for determining an uptake rate constant. The constant, although not directly applicable to environmental contamination models, is a valuable indication of an important plant process which, with other factors, determines chemical uptake into agricultural and wild plants.

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